Vasoactive Intestinal Peptide and the Watery Diarrhea Syndrome

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A sensitive and specific radioimmunoassay for the detection of vasoactive intestinal peptide has been used to study patients with the watery diarrhea syndrome. In eleven patients the syndrome was associated with tumors, and plasma levels of vasoactive intestinal peptide were elevated. VIP levels returned towards normal in five treated patients coincident with amelioration of symptoms. Normal values were obtained in patients with chronic pancreatitis, sprue, medullary carcinoma, Zollinger-Ellison Syndrome and laxative abuse. In six other patients with an indistinguishable syndrome and no findings of tumor at laparotomy and autopsy, vasoactive intestinal peptide levels were normal. The results suggest that VIP may be the causative agent in patients with the watery diarrhea syndrome and tumors, but that an indistinguishable syndrome exists for which VIP is not the cause.

THE ADVANCES IN PROTEIN chemistry and radioimmunoassay techniques over the past decade have resulted in the proliferation of a number of gut candidate hormones. One of the more interesting, more recently discovered candidate hormones is the 28 amino acid peptide, vasoactive intestinal peptide. This peptide derives its name from its most striking physiological characteristic, production of hypotension and profound vasodilatation when given systemically.[14] In addition, it has several other potent effects, among which are the inhibition of histamine and pentagastrin stimulated gastric secretion,[19] the stimulation of exocrine pancreas secretion[15] and the marked increase in small intestinal secretion.[2] The latter actions are similar to those physiological characteristics noted in the watery diarrhea or pancreatic cholera syndrome,[9,12,20,21] namely massive intestinal secretion, disturbances in glucose metabolism and inhibition of gastric secretion, which although not an essential part of the syndrome, reverse in some cases after adequate therapy.[10,17]

There have been several reports implicating vasoactive intestinal peptide as a mediator in the watery diarrhea syndrome.[3,7,16] Other reports, however, have not been conclusive,[7,12,17] and in other case reports as many as six hormones have been recovered from a single patient.[13,18]

In the following report, sera of various patients with the watery diarrhea syndrome were assayed for vasoactive intestinal peptide. The results indicate that whereas in most patients with the syndrome in association with tumors, plasma VIP levels are at least intermittently elevated. In several treated patients, plasma VIP levels return towards normal after appropriate therapy. In six patients, however, without tumors, an indistinguishable syndrome was not associated with elevated levels of vasoactive intestinal peptide. Vasoactive intestinal peptide may be a mediator, in some cases, of the watery diarrhea syndrome, but it apparently is not in several clinically indistinguishable other cases.

Methods

VIP was measured by a sensitive and specific radioimmunoassay technique previously described.[5] The antibody did not cross react with secretin, glucagon, cholecystokinin-pancreazymin, gastrin, pentagastrin, human growth hormone, insulin, calcitonin, GIP and parathyroid hormone in amounts up to 1000 ng/ml (Fig. 1).

Results

Normal Values

Normal levels of plasma vasoactive intestinal peptide were initially obtained in approximately 130 patients without known gastrointestinal disease. Mean

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values were 96 ± 4.1 pg/ml. Nineteen per cent of the population had values below 15 pg/ml, the least value which is accurately detectable by this radioimmunoassay. Approximately 17% of the population had values between 150–195 pg/ml. As we gained additional experience with the radioimmunoassay, values tend to become lower, as in the case with most evolving radioimmunoassays. In a more recent group of 50 patients and normal volunteers, without demonstrable gastrointestinal disease, values ranged between 22 and 70 pg/ml. The mean plasma VIP concentration was 42.8 ± 2.26 pg/ml.

Plasma Vasoactive Intestinal Peptide in Patients with a Watery Diarrhea Syndrome

Sera were obtained courtesy of Dr. J. Gardner, Dr. Robert M. Zollinger, Sr., Dr. Avram Cooperman and from patients treated at the Massachusetts General Hospital. In 11 patients with documented watery diarrhea syndrome and demonstrable tumors, either limited to the pancreas or metastatic to nodes and/or liver, levels in excess of 200 pg/ml were obtained. These ranged from 210 pg/ml to a high value of 2100 pg/ml in a patient previously reported by Kahn, et al.,[8] as case 1 (Table 1). In general, values were high enough so that overlap was not a problem, and ranged from 350–700 pg/ml (Table 1). Two patients had normal values of 39 and 36 pg/ml, but samples had been frozen for four to five years, and thawed several times. Other patients with diarrhea and other endocrine tumors were also assayed. In ten patients with the Zollinger-Ellison Syndrome and diarrhea, normal values were obtained. These ranged from 15–107 pg/ml. An additional patient with atypical pancreatic tumor morphology, the watery diarrhea syndrome, and a presumed gastrinoma, a value of 350 pg/ml was obtained. This patient died shortly after presentation with widespread metastases, and it is possible that the elevated vasoactive intestinal peptide concentration was causally related to the diarrhea as 800 pg VIP/gr tumor was recovered from the tumor. In pancreatic insufficiency with severe diarrhea, values of 30 pg/ml were obtained. In four patients with sprue, entirely normal levels were obtained as well. Three patients with laxative abuse had normal values as well (Fig. 2).

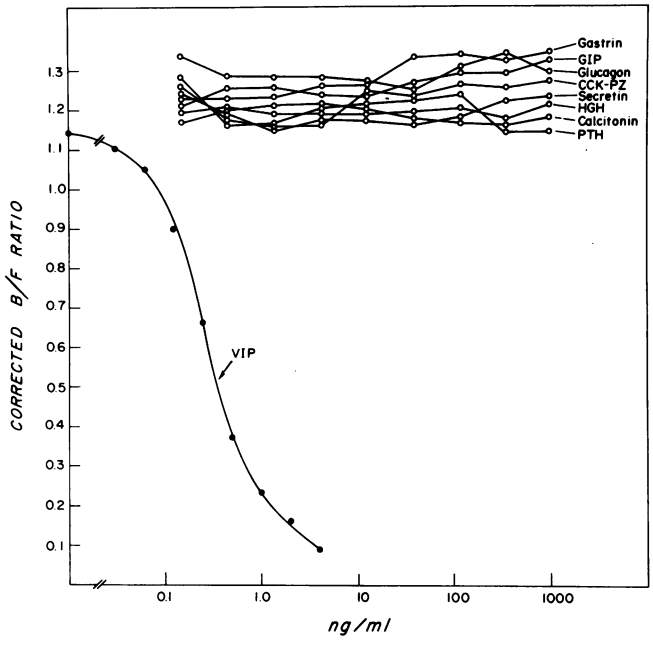

FIG. 1. Curve of current radioimmunoassay of vasoactive intestinal peptide. Note that the antibody does not apparently cross react with any of eight known gut peptides thus far treated.

TABLE 1. *Cases of Verner Morrison Syndrome with Elevated Plasma VIP*

Case #	Source	Plasma VIP	Pathology	Comments
1	RMZ	210	Non-B-Islet	
2	RMZ	91,430,290 22	Non-B-Islet	Preoperative Postoperative and Streptozotocin
3	JG	2100 1600	Non-B-Islet	Metastatic Post-streptozotocin
4	JG	395,172	Non-B-Islet	
5	JG	220,115,95 30	Non-B-Islet	Preoperative Postoperative
6	MGH	245 23	Non-B-Islet	Preoperative Postoperative Resection
7	MGH	696,705	Non-B-Islet	
8	MGH	410,382,340	Non-B-Islet	
9	MGH	645,537	Non-B-Islet	
10	JG	290 105	Non-B-Islet	Pre-streptozotocin Post-streptozotocin (Case #2—Kahn, et al.[14])
11	AC	800	Non-B-Islet	

Sources of patient sera: RMZ, Robert M. Zollinger, St., Ohio State University, J.G., Jerry Gardner, National Institutes of Health; AC, Avram Cooperman, Cleveland Clinic Foundation, Cleveland, Ohio; MGH, Massachusetts General Hospital.

Patients Assayed Before and After Therapy

Case 2 as reported by Kahn, et al. had an elevated level of approximately 290 pg/ml before therapy and which fell to 105 pg/ml after streptozotocin which ameliorated her symptoms considerably (Fig. 3).[8] Another patient had a fall from 245 pg/ml to 23 pg/ml following resection of an apparently single non-B-islet

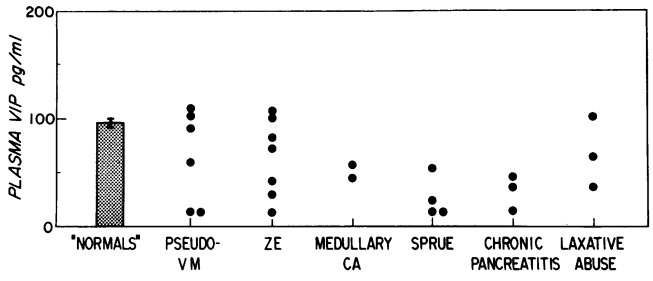

FIG. 2. Plasma vasoactive intestinal peptide levels in other conditions associated with chronic diarrhea, including the Zollinger-Ellison syndrome, medullary carcinoma of the thyroid sprue, chronic pancreatitis and laxative abuse. Although at the present time, very few patients without known gastrointestinal disease have plasma VIP levels of up to 100, the upper level of normal is taken at this level in the interest of avoiding overlap. Patients with a pseudo Verner Morrison Syndrome are separately described in the text.

cell tumor which resulted in remission of the symptoms. In another patient followed by Dr. R. M. Zollinger, Sr., plasma VIP fell from 290 to 22 pg/ml following resection and treatment of metastases with streptozotocin. In several other patients treated with streptozotocin, values fell, but in one, not to normal values (Fig. 3) at a time patients were in remission.

Other patients with the watery diarrhea syndrome. Sera from six patients who had an indistinguishable clinical syndrome including hypokalemia, massive volumes of diarrhea of up to 5 liters/day, hypochlorhydria, disturbances in glucose tolerance, and in some, disturbances in calcium metabolism were available. All of these patients underwent exploratory laparotomy following extensive work-up which failed to reveal the source of the syndrome. No findings were present in operation, and in some patients, blind distal pancreatectomy failed to ameliorate the symptoms of diarrhea. In one patient (see below) total pancreatectomy ameliorated diarrhea, but she did not survive long enough to establish this with certainty. All six patients ultimately died of metabolic disturbances or in one case (see below), a pulmonary embolus. In extensive postmortem examinations, no hyperplasia of the islets was found and no tumors or any organ demonstrated. VIP levels in these patients were all within the normal range, ranging from 20 pg/ml to 105 pg/ml (Fig. 2, Table 2). Two illustrative case histories of patients cared for at the Massachusetts General Hospital are included.

Case Reports

Case 1. C. G., Unit #141-62-85, Date of Admission: July 3, 1973. Date of Discharge: September 14, 1973 (death). Sudden onset of diarrhea and vomiting and crampy abdominal pain of approximately three to eight weeks duration. He gave a history of chronic pancreatitis, thought secondary to alcoholism. In 1966 and 1967 several operative procedures for pancreatitis were carried out, including drainage of multiple intraperitoneal abscesses and drainage of a pseudocyst. No alcoholic intake followed his 1967 admission. In 1972 there was brief episode of diarrhea which subsided spontaneously.

On physical examination, there were hyperactive bowel sounds and tenderness in the epigastrium and in the left lower quadrant. Various studies revealed only "gastroduodenitis" on gastroscopy and x-ray. There was no malabsorption. Ova and parasites were absent, and microscopic examination of stool showed no increased fats or muscle fibers. Gastrins were 210, 215 and 238 pg/ml on successive occasions, only slightly elevated. Acid studies ruled out Zollinger-Ellison Syndrome (0.6 mEq HCl/basal hr). 5-HIAA was normal (4.3 mg/24 hrs). Diarrhea persisted in volumes of up to 6 liter/day. A tendency to, but not frank hypokalemia was noted, requiring about 80 mEq K^+/24 hours. Studies of thyroid and adrenal functions were entirely within normal limits. Trials of nasogastric suction revealed large outputs (2000 ml/24 hours) of low acidity secretion. Notably, however, when he was on suction, diarrheal volume fell markedly. After numerous trials of therapy including steroids, cholestyramine and various opiates, on 8/24/73, abdominal exploration was undertaken. Positive findings consisted only of a nodule in the pancreas. A partial pancreatectomy was then carried out. Twenty-four hours postoperatively, the patient began to have explosive diarrhea again, between 1–5000 cc/24 hours, and remained a problem with massive gastrostomy outputs and/or massive diarrhea, with

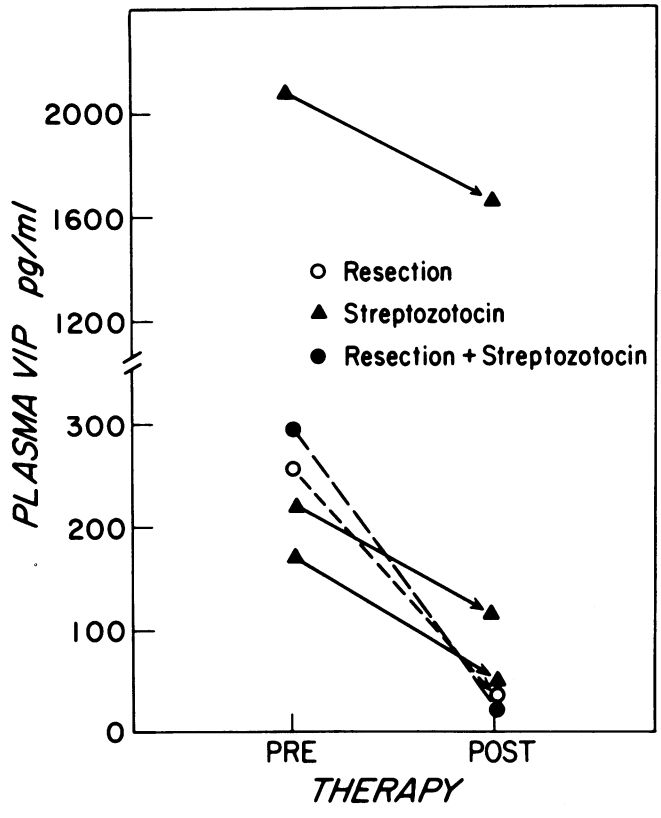

FIG. 3. The response of plasma VIP levels to therapy. In this group of five patients in whom samples have been assayed before and after therapy, there has been only one patient in whom resection has been carried out and who did not have residual disease. In this patient, all symptoms disappeared and levels fell to well within normal limits. In the other three patients symptoms were ameliorated at the time of posttherapy samples. In an additional, more recent patient (RMZ), levels decreased from 200–400 to normal following resection and streptozotocin for treatment of metastatic disease (Table 1, Case 2).

fever, dehydration and toxicity. He suddenly expired 14 days after surgery.

Postmortem examination revealed moderate pancreatitis in the residual head of the pancreas with a pseudocyst containing 1500 cc of fluid as well as retroperitoneal fat necrosis and fibrosis. There was small bowel edema and moderate dilatation and some peritonitis. There was also bilateral pulmonary edema. No tumor was found despite a careful search.

Case 2. A. G.. Unit #169-61-70. Date of Admission: March 9, 1971. Date of Discharge: August 21, 1971. The patient a 57-year-old woman suffered several weeks of explosive diarrhea eight years prior to her present admission. This subsided without any specific therapy. Two months before admission, she noted sudden onset of watery diarrhea of between 12–15 movements/day of 100–200 cc with little solid material. She had entered another hospital during which she required 4–5 liters/day of intravenous fluid with a tendency to hypokalemia and acidosis. Additional findings include a bilirubin of two, diabetes mellitus (a new finding), and mild anemia with PCV of 34%. Shortly after admission, she sustained a brief episode of renal failure which resolved in approximately five days. When a nasogastric tube was placed, it drained only 400–600 cc, but stool volume dropped from an average of about 3–4000 cc/24 hours to approximately 200–300 cc/24 hours. This was repeated several times in her course. Diarrhea continued unabated, and was complicated by two episodes of unresponsiveness and lethargy. Plasma gastrins, prostaglandins, serotonin and histamine were within normal limits. No malabsorption was present. Acid studies showed basal 0.6 mEq/hr, MAO 4.1 mEq/hr.

On 3/29/71, at laparotomy, the large and small bowel were fluid filled, the gall bladder was tense, and there was no ulcer, the pylorus was somewhat hypertrophied, no tumor was found in the adrenal. The only abnormality was a pancreas which was enlarged and firm with induration throughout its length, but specifically in the head. A pancreatoduodenectomy was carried out. Diarrhea resumed on the fourth postopeative day. The pathology report showed chronic pancreatitis with exocrine atrophy and squamous metaplasia of the pancreatic ducts. Diarrhea continued unabated and she was unresponsive to prednisone, indomethacin and gluten free diets. On 7/9/71, she underwent distal pancreatectomy and splenectomy. Following total pancreatectomy, she did well and was able to resume oral feedings with pancreatic supplements and without massive volumes of diarrhea. Unfortunately, she developed pyelonephritis and sustained a pulmonary embolus. On 8/19/71, she had a cardiac arrest from which she was resuscitated. However, she had a downhill course, failed to recover and expired.

Postmortem examination failed to reveal any evidence of additional tumor. Death was thought to be due to both sepsis in the blocked kidney and pyelonephritis, and a massive pulmonary embolus.

Subsequent assays revealed a plasma VIP of 40 pg/ml on multiple samples. Bioassay (courtesy Prof. T. Scratcherd) showed 600 pg/ml secretin-like activity prior to pancreateduodenectomy and absent activity postoperatively, although diarrhea was still present.

Comment

In both of these patients with massive diarrhea of up to 6000 cc/day, no tumor was ever found. In one patient, patient 2, secretin-like activity was recovered preoperatively from plasma and from the abnormal pancreas. In patient 1, no such assay was carried out. Both of these patients showed a tendency to decrease the diarrhea when gastric aspiration was employed. This has not been a feature of patients with tumors, to our knowledge. Both pancreases were abnormal, although there was no sign of pancreatic insufficiency on preoperative evaluation, with no increase in stool fat or nitrogen. The entire clinical picture was consistent with some secretory form of diarrhea, but plasma VIP was normal in both patients as well as four others (Table 2).

TABLE 2. *Pseudo Verner Morrison*

Case #	Source	Plasma VIP pg/ml	Comments
1	JG	91,30,115	
2	SB	<30	
3	SB	66	
4	MGH	<30,<30	Case 1
5	MGH	40,45	Case 2
6	AC	60	

Sources of patient sera: J.G., Jerry Gardner, National Institutes of Health, S.B., Steven Bloom, Hammersmith Hospital, London, MGH, Massachusetts General Hospital, A.C., Avram Cooperman, Cleveland Clinic Foundation.

Discussion

The watery diarrhea syndrome, originally confused as a variant of the Zollinger-Ellison Syndrome and associated with a non-B-islet cell tumor of the pancreas has been known for some time. The first clear demonstration of the separate syndrome is generally credited to Verner and Morrison,[20] although the report of Moldawer, et al.,[17] from this institution may have been an early case. Additional reports followed after Verner and Morrison, and made it clear that in contrast the patients with the Zollinger-Ellison Syndrome, these patients did not manifest elevated levels of gastric secretion, but hypochlorhydria.[12] Matsumoto, et al. coined the term "pancreatic cholera" to emphasize that the causation of the syndrome was often a tumor of the pancreas[10] and Marks, et al.[9] suggested the "WDHA Syndrome" to point out the salient characteristics of massive diarrhea, hypokalemia and hypochlorhydria. It is now generally agreed that achlorhydria is not a necessary part of the clinical syndrome, but that hypochlorhydria or even normal acid levels may exist. What is clear is that in some of the cases which have been reported, gastric secretion returned to normal or elevated levels following resection of the presumably inhibitory tumor.[9,10,17]

A number of suggestions have been made for the presumed hormone mediator of this syndrome, including secretin,[21] GIP[6] and gastrin and glucagon.[1] Plasma gastrins have been normal in most of the patients assayed to date. Bloom and his coworkers[3] and Said and Faloona[16] have reported six and 28 cases, respectively of the watery diarrhea syndrome, suggesting that the chemical mediator was vasoactive intestinal peptide. Other case reports have proven somewhat confusing. Thus, while Kahn, et al.,[8] case 1 had high plasma levels measured by Said, the plasma VIP remained at its previous level following therapy at a time when the patient's clinical syndrome was ameliorated. Case 2 had no detectable VIP by another radioimmunoassay,[8] but did have an elevated level which responded to treatment as detected by this radioimmunoassay. Other papers have focused on the multiplicity of hormones, including calcitonin, prostaglandins, increased urinary excretion of gastric secretagogue activity and tetrahydraldosterone were reported by Rambaud, et al..[13] Schmitt et al.[8] attributed their case of the watery diarrhea syndrome to secretin, although Bloom failed to detect elevated levels in this patient, a discrepancy attributed to poor storage. Serotonin, enteroglucagon and pancreatic glucagon were also recovered from the plasma of this patient. This report differs from others reported in that the site of excess secretion appeared to be the pancreas rather than in the small bowel.[18]

In this report, eleven patients with the watery diarrhea syndrome had elevated levels of vasoactive intestinal peptide at some time during their course, while patients with other causes for diarrhea, including various other endocrine tumors (with the exception of one patient with a gastrinoma who may have been secreting VIP as well), sprue or medullary carcinoma of the thyroid have normal levels of vasoactive intestinal peptide. In five patients, amelioration of their symptoms appeared to coincide with a decrease in plasma levels of vasoactive intestinal peptide following successful therapy. VIP has been recovered from several tumors made available to us by Dr. Avram Cooperman at the Cleveland Clinic in amounts of up to 700 ng/gm of tissue. However, not all samples from patients with the watery diarrhea syndrome and non-B-islet tumors have elevated levels of VIP in plasma, despite histological confirmation of tumor and apparent clinical remission following resection. It is possible that intermittent secretion of these tumors of VIP occurs, something in keeping with clinical variations in activity. Other possibilities include different molecular species of VIP or, of course, the possibility that VIP is not the mediator in these patients. Tests of jejunal or pancreatic secretion or absorption are not available in most of these patients, and the type of secretory diarrhea, therefore, is not clear.

This report also makes clear, however, that an indistinguishable clinical syndrome may be associated with normal levels of vasoactive intestinal peptide in patients well-studied in various institutions, and who have come to thorough postmortem examination without finding any evidence of tumor. In one of these patients, elevated levels of secretin-like activity was detected on bioassay (case 2), but the nature of this particular increase in secretin-like activity is not clear at the present time. It is possible that secretin is a mediating hormone in this patient, since it does appear, as in Schmitt's case, that diarrhea decreased markedly when nasogastric aspiration, especially in the duodenum was instituted. Extensive radioimmunoassays have been carried out on samples of some of the other patients without any positive findings, suggesting the etiology of some of the cases of the watery diarrhea syndrome may be due to another as yet unknown substance.

Taken together, the results clearly suggest that while vasoactive intestinal peptide may be the mediator of one form of "pancreatic cholera" or watery diarrhea syndrome, another group of patients with non-B-islet cell tumors have normal level of VIP, at least in the plasma samples available to us. Furthermore, from recent investigations using intestinal perfusion techniques, it appears likely that sites of secretion differ in various patients, and that at least two forms of secretory diarrhea exist, one of pancreatic, the other of jejunal origin (Fordtran, and Soergel, personal communication). Presumably, these two secretory patterns reflect differing etiologic agents. An indistinguishable group of patients in whom no tumor will be found, no matter how thorough the search, failed to demonstrate elevated levels of vasoactive intestinal peptide nor any source of their syndrome at postmortem examination. Bloom and Polak[4] have studied eight such patients and suggested "Pseudo Verner Morrison Syndrome" for this group. The etiology of their watery diarrhea at the present time remains unknown.

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